学位論文

論　文　題　目

Fluvastatin-induced reduction of oxidative stress ameliorates diabetic cardiomyopathy in association with improving coronary microvasculature.

糖尿病性心筋症に対するフルバスタチン長期投与の効果
―抗酸化作用および冠微小循環から見た検討―

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ABSTRACT

Diabetic cardiomyopathy is associated with increased oxidative stress and vascular endothelial dysfunction, that lead to coronary microangiopathy. We tested whether statin-induced redox imbalance improvements could ameliorate diabetic cardiomyopathy and improve coronary microvasculature in streptozotocin-induced diabetes (DM). Fluvastatin (10 mg/kg/day) or vehicle was orally administered for 12 weeks to rats with or without DM. Myocardial oxidative stress was assessed by NADPH oxidase subunit $p22^{phox}$ and $gp91^{phox}$ mRNA expression and myocardial 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) levels. Myocardial vascular densities were assessed using anti-CD31 and anti-α smooth muscle actin (SMA) antibodies. Fluvastatin did not affect blood pressure or plasma cholesterol, but attenuated increased left ventricular (LV) minimum pressure and ameliorated LV systolic dysfunction in DM rats compared to vehicle (LVdP/dt, 8.9±1.8 vs. 5.4±1.0 $\times 10^3$ mmHg/s, $p<0.05$). Myocardial oxidative stress increased in DM, but fluvastatin significantly reduced $p22^{phox}$ and $gp91^{phox}$ mRNA expression and myocardial $PGF_{2\alpha}$ levels. Fluvastatin enhanced myocardial endothelial NO synthase (eNOS) protein levels and increased eNOS, vascular endothelial growth factor, and hypoxia-inducible factor-1 α mRNA expression. CD31-positive cell densities were

lower in DM rats than in non-DM rats (28.4±13.2 vs. 48.6±4.3/field, $p<0.05$) and fluvastatin restored the number (57.8±18.3/field), although there were no significant differences in SMA-positive cell densities between groups. Fluvastatin did not affect cardiac function, oxidative stress, or vessel densities in non-DM rats. These results suggest that beneficial effects of fluvastatin on diabetic cardiomyopathy might result, at least in part, from improving coronary microvasculature through reduction in myocardial oxidative stress and angiogenic factor up-regulation.

Keywords

Streptozotocin, statin, oxidative stress, coronary microvessels

INTRODUCTION

Patients with diabetes are often associated with reduced cardiac function despite the absence of coronary atherosclerotic disease, i.e., diabetic cardiomyopathy. Oxidative stress in diabetes plays a key role in the pathogenesis of diabetic cardiomyopathy (1, 2). Hyperglycemia can stimulate the production of reactive oxygen species (ROS) from a variety of sources (3-6), and affect cardiac and endothelial function. Clinical studies have demonstrated consistent abnormalities in the coronary flow reserve of patients with diabetes in the absence of epicardial coronary artery disease (7-9), while animal studies have shown that hearts in the presence of diabetes are associated with an impaired angiogenic capacity and reduced microvessel densities (10, 11). Therefore, therapeutic interventions that aim to reduce oxidative stress and/or stimulate angiogenesis could inhibit the progression of diabetic cardiomyopathy.

Statins, a class of drugs used to lower blood cholesterol by inhibiting biosynthetic enzymes in the liver, effectively reduce the risk of cardiovascular events in patients with diabetes (12, 13). The cardioprotective effects of statins, independent of their lipid-lowering-effect, could result primarily from increased NO bioavailability through the activation of endothelial NO synthase (eNOS) and enhancement of eNOS mRNA stability (14, 15). Statin-induced eNOS increases and oxidative stress reductions inhibit intramyocardial inflammation (13, 16).

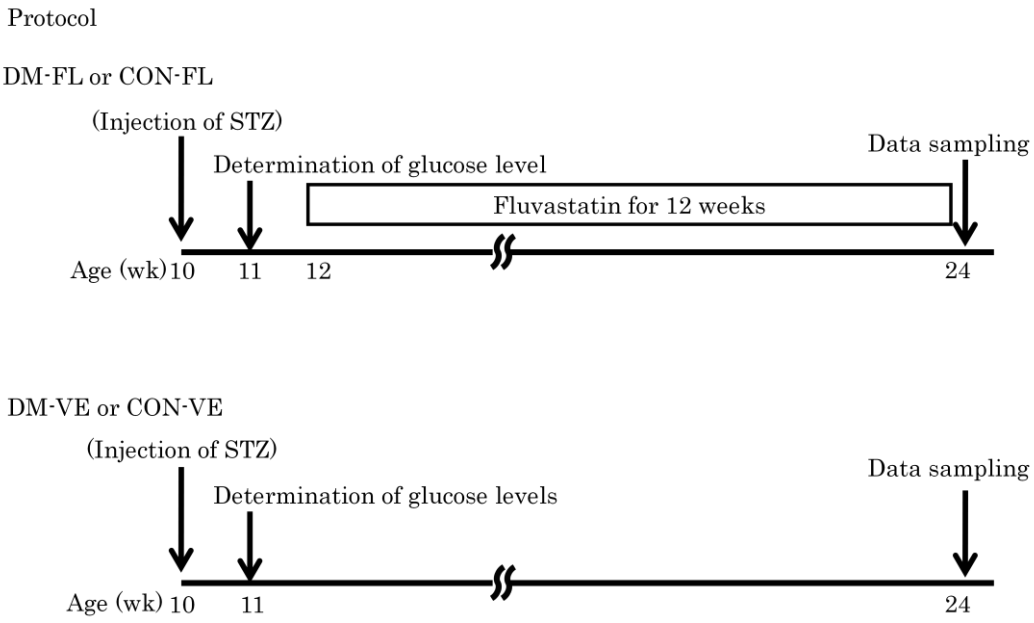
Moreover, statins mobilize endothelial progenitor cells from the bone marrow, while NO is a stimulator of angiogenesis (17). Therefore, the purpose of the present study was to investigate whether fluvastatin, which has a powerful antioxidative effect (18), could inhibit the progression of diabetic cardiomyopathy by reducing myocardial oxidative stress and its proangiogenic action.

MATERIALS AND METHODS

This study proceeded in accordance with the University of Toyama guidelines for animal experiments.

Experimental animals

Diabetes was induced in male 10-week-old Wistar rats by an intraperitoneal injection of 65 mg/kg of streptozotocin (STZ). Control rats without diabetes were not injected with STZ. The rats with glucose levels of > 300 mg/dL one week after the STZ injection were considered to have diabetes mellitus (DM) and were used in the following experiments. Starting 2 weeks after the STZ injection, fluvastatin (10 mg/kg/day) was orally administered to the rats by gavage for 12 weeks. Standard rat chow and tap water were provided ad libitum throughout the study.



Echocardiographic and hemodynamic studies

Transthoracic echocardiography was performed using an ultrasonography system equipped with a 7.5-MHz transducer (SONOLAYER SSA-260A, Toshiba, Tokyo, Japan) at the end of the study. Left ventricular end-diastolic (LVDd) and left ventricular end-systolic (LVDs) diameters were determined under light anesthesia with sodium pentobarbital (15 mg/kg, i.p.). After the echocardiographic study, a 2F micromanometer-tipped catheter was introduced through the right carotid artery and advanced to the left ventricle (LV) to determine LV systolic pressure (LVSP), minimum pressure, and maximal and minimal rates of LV pressure changes (LV dP/dt_{max} and LV dP/dt_{min}, respectively).

Quantitative real-time reverse-transcriptase polymerase chain reaction (PCR)

The procedures for quantitative real-time reverse transcription PCR were described previously (19). Briefly, total RNA extracted from 100 mg of LV tissue using Isogen (Nippon Gene, Tokyo, Japan) was digested with DNase (Takara Bio, Shiga, Japan) to eliminate genomic DNA contamination. RNA samples were reverse transcribed with oligo (dT) primers using an RNA PCR kit (Takara Bio, Shiga, Japan). Quantitative PCR analysis proceeded using a sequence detector (Mx3000P; Agilent Technologies, Santa Clara, CA, USA) in a total volume of 20 μL containing 1 μL of cDNA, 10 μL of reagent (Brilliant II Fast QPCR MasterMix; Agilent Technologies), 8 μL of diethylpyrocarbonate-treated water and 1 μL of primer and TaqMan probe sets (Applied Biosystems, CA, USA) specific for cDNAs encoding glyceraldehyde-3-phsosphate dehydrogenase (GAPDH), vascular endothelial growth factor A (VEGF), hypoxia-inducible factor-1α (HIF-1α), nitric oxide synthase 3 endothelial cell (eNOS), cytochrome b-245 alpha polypeptide (p22), cytochrome b-245 beta polypeptide (gp91), and transforming growth factor beta 1 (TGF-β1). The PCR program comprised 40 cycles of denaturation at 95°C for 1 min, primer annealing at 40°C for 5 sec, and extension at 60°C for 20 sec.

Immunoblotting

Myocardial proteins were immunoblotted to determine rat eNOS protein levels, as described previously (19). Tissue samples (20 μg of protein) were

resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 5 -15% gels (Ready Gel J; Bio-Rad Laboratories, Tokyo, Japan) and the separated proteins were transferred to a polyvinylidene difluoride membrane (Immun-Blot PVDF Membrane for Protein Blotting [0.2 μm]; Bio-Rad, Tokyo, Japan). The membrane was incubated at room temperature for 1 hour with 5% skim milk and 0.1% Tween-20 in Tris-buffered saline and then overnight with rabbit polyclonal antibodies to eNOS (eNOS antibody; Cell Signalling Technologies, Beverly, MA, USA) at a 1:1000 dilution in the same solution. The membrane was washed and incubated for 1 hour with a 1:1000 dilution of horseradish peroxidase-conjugated goat antibodies to rabbit IgG (anti-rabbit IgG HRP-linked antibody; Cell Signalling Technologies, Beverly, MA, USA). The intensity of the eNOS bands was quantified using densitometry (LAS-4000; Fujifilm, Tokyo, Japan).

Cardiac PGF2α levels

The procedures for the determining of cardiac PGF2α were described previously (20). Briefly, LV tissues were disrupted in a Polytron Homogenizer with 50 mM HCl and the homogenate was centrifuged for 5 min. The supernatant was extracted with ethyl acetate by centrifugation at 3,000 rpm for 5 min and the resulting organic layer was evaporated under a nitrogen stream. The residue was dissolved in a mixture (50 μL) of acetonitrile and ethanol, and 1 mM HCl (2.5 mL) was then added. Tissue extract was applied to Empore Disk Cartridges

pre-conditioned with methanol and 1 mM HCl. The cartridges were washed with 1 mM HCl followed by heptane. We eluted PGF2α with ethyl acetate containing 1% methanol, and evaporated the eluate under a steam of nitrogen. The residue was dissolved in 0.05% formic acid and acetonitrile, while the PGF2α level was determined using high performance liquid chromatography–electrospray ionization–mass spectrometry (API 4000™ LC/MS/MS; Applied Biosystems) (21). In the present study, we determined PGF2α levels in rats with DM but not in rats without DM, because the PGF2α level in rats without DM was below the detection limit (0.1 ng/g tissue) in our previous study (20).

Histology

The LV was cut into transverse slices, fixed in 10% formaldehyde, embedded in paraffin, and cut into 5-μm-thick sections for staining with Sirius Red to determine the fibrosis area. Using an image analyzer (MetaMorph Image Analysis offline version 7.1.1.0), 4 fields at the level of the papillary muscle were randomly chosen at a magnification of ×400. The fibrosis area was determined by an investigator who was unaware of the experimental groups.

Immunohistochemical analysis

Portions of the transversely cut LV specimens were immunohistochemically examined to determine coronary arteriolar and capillary densities. Vascular smooth muscle and endothelial cells were detected by overnight incubation with

antibodies to α-smooth muscle actin (α-SMA M0851; Dako, Tokyo, Japan) and CD31 (PECAM-1; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), respectively. The specimens were then incubated with biotinylated anti-rabbit IgG. Arteriolar densities were determined from the number of α-SMA-positive microvessels with an internal diameter of <50 μm and ≥1 layer of smooth muscle cells at a magnification of ×400. Capillary density was similarly determined from the number of CD31-positive vessels with an internal diameter of <10 μm at a magnification of ×800. Sixteen fields of the LV wall were randomly selected from each section and counted by an investigator who was unaware of the experimental groups

Statistics

Results are expressed as means ± SD. Differences among groups were tested using a one-way analysis of variance followed by the Bonferroni test for multiple comparisons. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Hemodynamics

Plasma blood glucose and triglyceride levels were markedly elevated in rats with DM. Fluvastatin did not affect blood glucose or total cholesterol levels in rats with or without DM but attenuated the elevated triglyceride levels in rats with DM (Table 1).

The body and heart weights of rats with DM were lower than those without DM, but the heart-to-body weight ratio was significantly greater in rats with DM (Table 2). Rats with DM had elevated LV minimum pressure that was decreased by fluvastatin to the level seen in rats without DM. Both maximum and minimum LVdP/dt values were reduced in rats with DM, but fluvastatin restored these values, although the significance of the improvement in minimum LVdP/dt was only borderline (p=0.089). Echocardiographic assessment showed that LV fractional shortening was lower in rats with DM than in rats without DM, but was not significantly changed by fluvastatin.

Table 1. Blood glucose and plasma lipid levels

	Non-DM		DM	
	Vehicle (n=7)	Fluvastatin (n=7)	Vehicle (n=6)	Fluvastatin (n=7)
BG (mg/dL)	168±73	170±66	414±41**	490±208**
T-CHO (mg/dL)	82±9	74±12	104±21	92±21
TG (mg/dL)	32±19	26±5	226±67**	129±73**##
NEFA (mEq/L)	0.57±0.10	0.67±0.19	1.19±0.31**	0.87±0.20

Values are means ± SD. ** p<0.01 vs. vehicle of non-diabetes mellitus (DM); ## p<0.01 vs. vehicle of DM. BG indicates blood glucose; T-CHO, total cholesterol; TG, triglyceride; NEFA, non-esterified fatty acids.

Table 2. Hemodynamic data

	Non-DM		DM	
	Vehicle (n=7)	Fluvastatin (n=7)	Vehicle (n=6)	Fluvastatin (n=7)
BW (g)	467±14	450±26	242±60**	271±66**
HW (mg)	1114±80	1061±116	869±170*	851±172*
HW/BW (mg/g)	2.38±0.16	2.36±0.17	3.65±0.52**	3.19±0.42**
HR (beats/min)	383±39	395±27	351±31	370±15
Systolic LV P (mmHg)	124±11	114±14	115±13	120±13
Minimum LVP (mmHg)	0±1	-2±2	7±8*	-1±1##
LV dP/dt_{max} ($x10^3$ mmHg/s)	9.69±2.23	9.56±1.64	5.42±1.03**	8.92±1.87#
LV dP/dt_{min} ($x10^3$ mmHg/s)	-8.05±0.64	-7.84±1.70	-4.71±0.70**	-6.52±1.48
LVDd (mm)	5.0±0.71	5.19±0.35	5.69±0.51	5.84±0.49
LVDs (mm)	1.67±0.56	1.96±0.42	2.86±0.45**	3.24±0.36**
%FS	66.9±7.8	64.4±7.5	48.8±6.1**	44.2±5.2**

Values are means ± SD. * $p<0.05$ and ** $p<0.01$ vs. vehicle of non-diabetes mellitus (non-DM). # $p<0.05$ and ## $p<0.01$ vs. vehicle of DM. BW indicates body weight; HW, heart weight; HR, heart rate; LVP, left ventricular pressure; LV

dP/dtmax and LV dP/dtmin, peak rate of LVP rise and fall, respectively; LVDd and LVDs, LV end-diastolic and end-systolic dimension, respectively; FS, fractional shortening.

Oxidative stress in the myocardial tissue

The expression of NADPH oxidase subunit $p22^{phox}$ and $gp91^{phox}$ in the myocardial tissue of rats with DM was significantly higher than that of rats without DM, while fluvastatin attenuated the upregulated expression of these subunits in rats with DM (Figure 1). Similarly, cardiac levels of PGF2α were markedly low in fluvastatin-treated rats with DM, compared to rats with DM without fluvastatin treatment.

Cardiac microvessel density and eNOS, VEGF and HIF-1α expression

The capillary density of LV assessed by CD31-positive cells was lower in rats with DM than in those without DM, but fluvastatin restored this value in rats with DM. The arteriole densities were not significantly different among the 4 groups (Figure 2). Fluvastatin significantly increased both VEGF and HIF-1α expression in the myocardium of rats with and without DM. In rats with DM, eNOS mRNA and its protein levels were also up-regulated by fluvastatin (Figure 3). The level of VEGF mRNA was correlated with eNOS ($r=0.506$, $p<0.05$) and HIF-1α ($r=0.647$, $p<0.01$) expression.

Cardiac fibrosis

Myocardial interstitial fibrosis was greater in rats with DM than in those without DM. Fluvastatin inhibited DM-induced increases in interstitial fibrosis (Figure 4). The up-regulated expression of TGF-β1 mRNA in rats with DM was also attenuated by fluvastatin treatment.

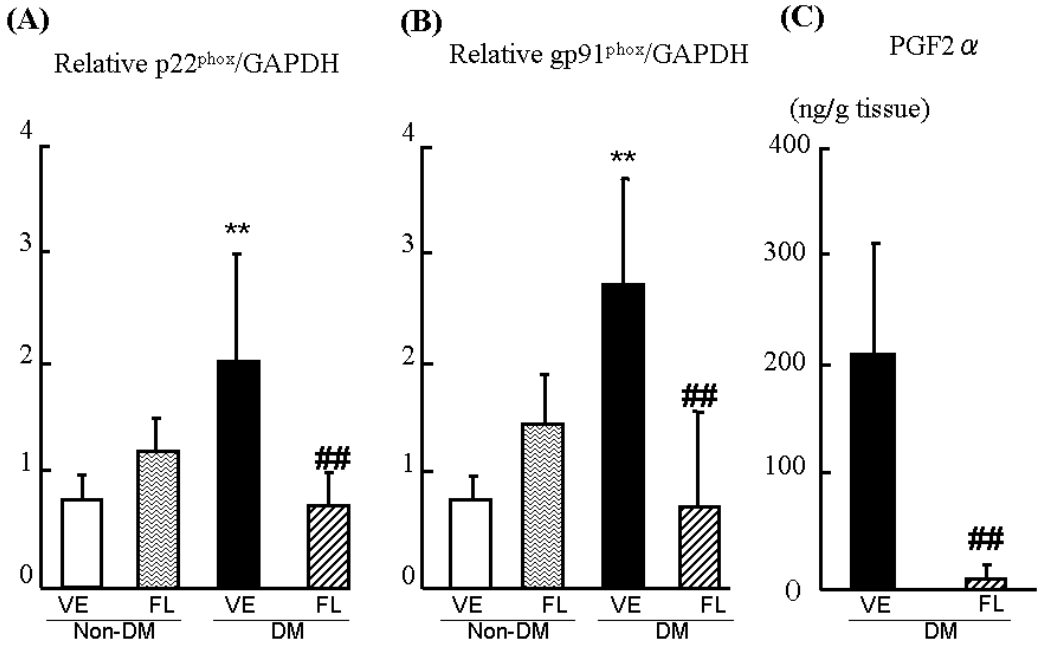


Figure 1. Left and middle panels, expression of myocardial NADPH oxidase subunit $p22^{phox}$ and $gp91^{phox}$ mRNA in rats without diabetes mellitus (non-DM rats) treated with vehicle (VE, n = 5) and fluvastatin (FL, n = 5) and in rats with DM

treated with VE (n = 5) and FL (n = 4). Right panel, myocardial 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) levels in rats with DM (DM rats) treated with VE (n = 5) and FL (n = 4). Values are means + SD. ** $p < 0.01$ vs. VE of non-DM rats and ## $p < 0.01$ vs. VE of DM rats.

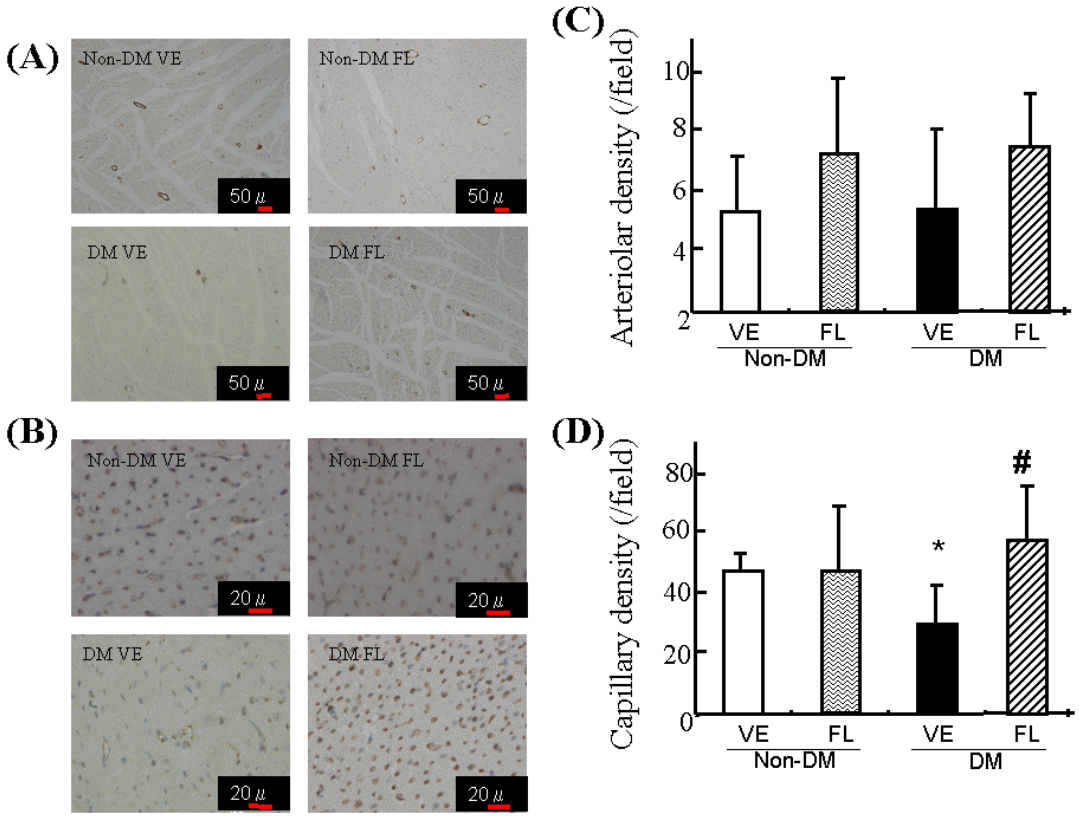


Figure 2. Representative photomicrographs of arterioles stained with α-smooth muscle actin (panel A) and capillaries with CD31 (panel B) in rats without diabetes mellitus (non-DM rats) treated with vehicle (VE) and fluvastatin (FL) and in rats with DM (DM rats) treated with VE and FL. Panels C and D show a quantitative morphometric analysis of arterioles and capillaries, respectively, in non-DM rats treated with VE (n = 5) and FL (n = 4) and in DM rats treated with VE (n = 5) and FL

(n = 5). Values are means + SD. *p < 0.05 vs. VE of non-DM rats. # p < 0.05 vs. VE of DM rats.

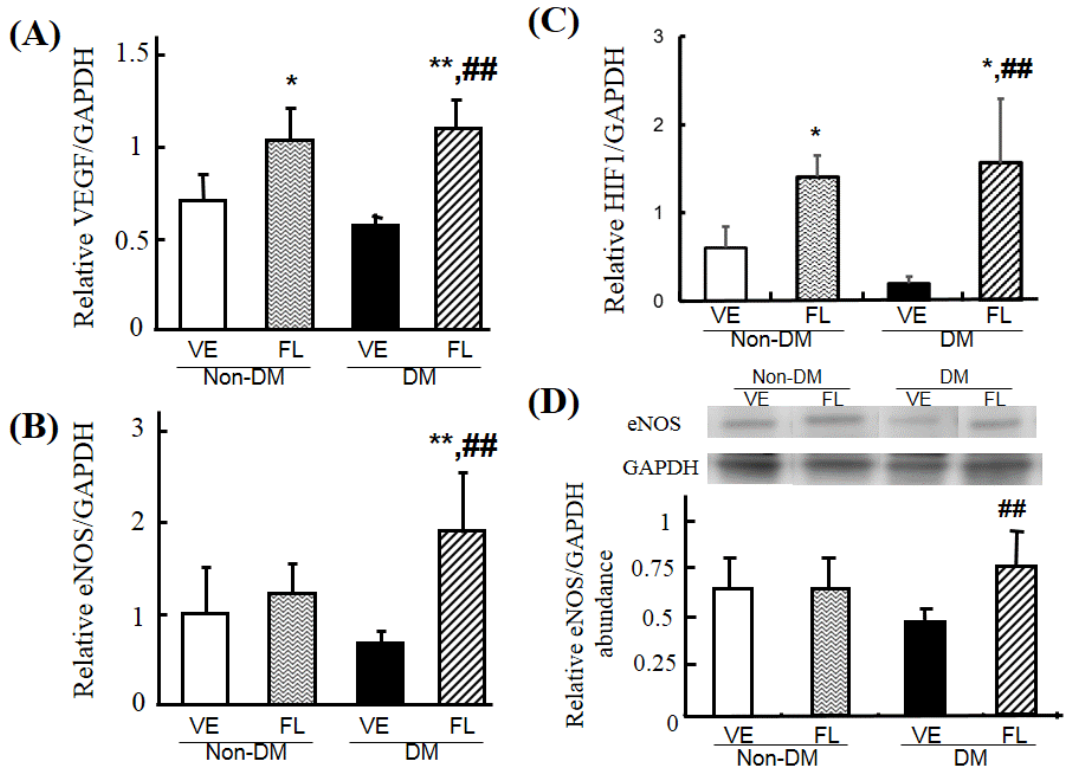


Figure 3. Panels A, B, and C show vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS), and hypoxia-inducible factor (HIF)-1α gene expression in rats without diabetes mellitus (non-DM rats) treated with vehicle (VE, n = 5) and fluvastatin (FL, n = 5) and in rats with DM (DM rats) treated with VE (n = 5) and FL (n = 4). Each mRNA level was normalized to the amount of GAPDH mRNA. Panel D shows representative examples of western blots for eNOS

and a densitometric analysis of eNOS protein expression in non-DM rats treated with VE (n = 5) and FL (n = 5) and in DM rats treated with VE (n = 5) and FL (n = 4). Values are means + SD. ** $p < 0.01$ vs. VE of non-DM rats. ## $p < 0.01$ vs. VE of DM rats.

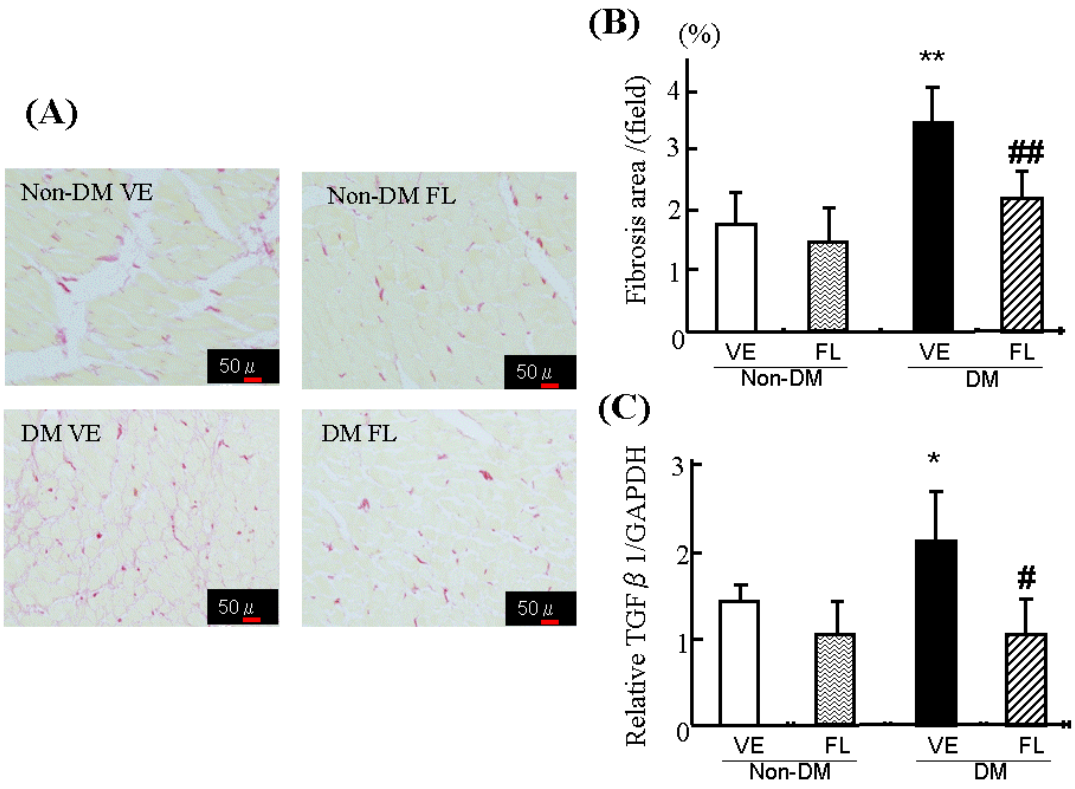


Figure 4. Panel A shows representative photomicrographs stained with Sirius Red in rats without diabetes mellitus (non-DM rats) treated with vehicle (VE) and fluvastatin (FL) and in rats with DM (DM rats) treated with VE and FL. Panel B shows the quantitative morphometric analysis of fibrosis areas in non-DM rats treated with VE (n = 5) and FL (n = 5) and in DM rats treated with VE (n = 5) and FL (n = 4). Panel C shows transforming growth factor-β1 (TGF-β1) gene expression in

non-DM rats treated with VE (n = 5) and FL (n = 5) and in DM rats treated with VE (n = 5) and FL (n = 4). Values are means + SD. * $p < 0.05$ and ** $p < 0.01$ vs. VE of non-DM rats. # $p < 0.05$ vs. VE of DM rats.

DISCUSSION

The major findings of the present study are as follows. First, a 3-month treatment with fluvastatin attenuated LV dysfunction in rats with STZ-induced DM. Second, fluvastatin suppressed the elevation of myocardial oxidative stress in rats with DM. Finally, fluvastatin restored the reduced myocardial capillary density in rats with DM in association with the upregulation of myocardial eNOS, VEGF, and HIF-1 α expression. These results suggest that the fluvastatin-induced LV dysfunction improvements might be a result, at least in part, of improved coronary vascularity in association with reduced myocardial oxidative stress in rats with DM.

LV dysfunction in rats with DM

STZ-induced DM is a well established model for the study of diabetic cardiomyopathy (22). The mechanisms involved in diabetic cardiomyopathy are not completely understood, but various factors might be involved in the progression of diabetic cardiomyopathy (23-25), including hyperglycemia (26), dyslipidemia (27), the production of inflammatory cytokines (28), increased oxidative stress (1, 2), and impaired coronary microcirculation (10, 11).

Non-atherosclerotic cardiomyopathy independent of vascular complications occurs in roughly 30% of all patients with type I DM and presents with early diastolic abnormalities followed by a later decline in systolic and overt heart failure (29). In the present study, LV dP/dt and LV fractional shortening were reduced, while minimum LV pressure was increased in rats with DM 12 weeks after the STZ injection, along with increases in myocardial oxidative stress and fibrosis but with myocardial capillary density reductions. Fluvastatin attenuated these STZ-induced deteriorations except for LV fractional shortening, probably because LV fractional shortening is load-dependent and is determined only from changes in the minor axis of the LV chamber.

Oxidative stress in DM rats

Studies have shown that oxidative stress is increased in both type I and type II DM (30, 31). Hyperglycemia induces oxidative stress through several pathways (3, 5, 6), including enhanced aldose reductase activity (6), increased advanced glycation end products (3), and altered protein kinase C activity (4). In the present study, rats with STZ-induced DM demonstrated increased myocardial oxidative stress as shown by increased NADPH oxidase subunit $p22^{phox}$ and $gp91^{phox}$ expression. The NADPH oxidase enzyme is an important source of ROS in vascular cells such as smooth muscle and endothelial cells (32, 33). F2 isoprostanes are a family of prostaglandin F2α isomers that are produced in vivo primarily from

oxidative modification of polyunsaturated fatty acids via a free radical-catalyzed mechanism. PGF2α is a major F2 isoprostane and was shown to be a specific and sensitive index of oxidative stress in vivo (34, 35). We demonstrated previously that cardiac PGF2α levels are markedly increased in rats with STZ-induced DM (20).

Long-term treatment with fluvastatin attenuated STZ-induced LV dysfunction in association with decreases in both cardiac PGF2α and NADPH oxidase subunit $p22^{phox}$ and $gp91^{phox}$ mRNA expression. In situations involving weakened antioxidant defence, superoxide anion will scavenge NO and lower NO bioavailability, leading to increased ROS production and inflammation (36). Excess oxidative stress might interfere with the VEGF signaling in DM (37). Statins decrease free radical generation in the vascular wall and myocardium (16, 38), while fluvastatin exerts potent antioxidant activities as a free radical scavenger—a property that is derived from its unique chemical structure (18, 39). Thus, in the present study, fluvastatin reduced cardiac oxidative stress in rats with DM, a finding consistent with that of an earlier study (20).

Coronary vascularity

Endothelial NOS was found to be dysfunctional due to reduced bioavailability of tetrahydrobiopterin (BH4) (40), while VEGF was downregulated in animals with STZ-induced DM (11, 41). The migratory and NO-releasing capacity

of endothelial progenitor cells (EPCs) was impaired by DM (11, 42). Thus, angiogenic factors of VEGF and eNOS are downregulated, which in turn hampers vessel formation in ischemic tissues.

Statins promote angiogenesis in ischemic limbs of animals (14). Nitropravastatin, an NO-releasing pravastatin derivative, stimulated reparative neovascularization and improved the recovery from limb ischemia in mice with STZ-induced DM (43). Statins facilitate the protein kinase Akt–eNOS interaction, promote eNOS activation/phosphorylation and NO-mediated angiogenesis, and mobilize EPCs from the bone marrow (17). The pro-angiogenic effect of statin may be ascribed to the activation of phosphatidylinositol 3-kinase (PI3K) -Akt pathway, and NO up-regulates the VEGF gene expression through a PI3K-Akt pathway, followed by induction of several transcription factors, especially HIF-1α (44, 45). In the present study, fluvastatin-induced up-regulation of VEGF was parallel to fuluvastatin-induced changes in HIF-1α expression. On the other hand, VEGF initiates the signaling cascades leading to NO production and angiogenesis (46). Thus the full pathways of fluvastatin-induced improvement of capillary density in DM remain to be elucidated, but the fluvastatin-induced up-regulation of eNOS and activation of VEGF signaling mediated by HIF-1α might be involved.

Microvascular abnormalities in patients with DM may lead to reduced

perfusion and mismatch of myocardial supply and demand. These microvascular changes may lead to ischemia in the absence of coronary atherosclerosis and contribute to adverse cardiovascular events in the DM patient (10, 47). A non-atherogenic cardiomyopathy occurs in roughly 30% of all type 1 DM patients (29), and pharmacological interventions for restoring coronary microvascular abnormalities may inhibit developing diabetic cardiomyopathy and adverse cardiovascular events.

Limitations

The present study had several limitations. First, the beneficial effects of fluvastatin on diabetic cardiomyopathy might be a result, at least in part, of improved coronary microvasculature by reducing myocardial oxidative stress and eNOS and VEGF up-regulation, but other factors such as the statin-induced suppression of inflammation or free radical generation may also contribute to improved diabetic cardiomyopathy (38, 48, 49). Fluvastatin up-regulated myocardial eNOS mRNA and its protein levels, but the phosphorylation of eNOS was not evaluated in the present study. The phosphorylation of eNOS might be important in angiogenesis and vascular homeostasis. Second, the correlation of myocardial capillary densities with eNOS or VEGF levels was not analysed because the animals that determined capillary densities were different from those for PCR or immunoblotting. Third, we did not determine the dose-response effect of

fluvastatin for protection of STZ-induced cardiomyopathy. In our previous studies, a 2-week treatment with fluvastatin (10 mg/kg/day) attenuated ischemia-reperfusion injury of non-diabetic rat heart (50), and this dose of fluvastatin reduced myocardial oxidative stress in rats with STZ-induced DM (20). Hayashidani et al. (51) reported that plasma concentration of fluvastatin in mice treated at the oral dose of 10 mg/kg/day was within the range of humans achieved after oral administration of its clinical doses (10 to 20 mg). According to these studies, we performed the study in the present dose of fluvastatin. Fluvastatin had dose-dependent free radical scavenging action in vitro (52), whereas low-dose statins were more effective than high-dose statins for augmenting collateral flow recovery in the ischemic limbs of ApoE-deficient mice (53). The influence of low- or high-dose fluvastatin on diabetic cardiomyopathy remains to be elucidated. Finally, it remains unknown whether statins other than fluvastatin have similar beneficial effects on diabetic cardiomyopathy. Van Linthout et al (48) showed that atorvastatin decreased intramyocardial inflammation and myocardial fibrosis, resulting in improved LV function in rats with DM, although they did not examine coronary vasculature. Sata et al (53) reported that all statins including cerivastatin, pitavastatin, and fluvastatin augmented capillary formation in ischemic limbs of mice without DM. Therefore, statins other than fluvastatin might have also promoted cardiac angiogenesis in animals with DM.

CONCLUSION

Long-term fluvastatin treatment attenuated cardiac dysfunction in rats with STZ-induced DM. This beneficial effect of fluvastatin might have resulted, at least in part, from the attenuation of DM-induced deterioration within the coronary microvasculature in association with reduced cardiac oxidative stress. The present results suggest that long-term treatment with statin might be beneficial to patients with diabetic cardiomyopathy.

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